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Recent knowledge on *Flavobacterium psychrophilum* in Denmark

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Rainbow trout fry syndrome (RTFS) or bacterial cold-water disease (CWD) has since the mid 1980s caused serious losses in the production of rainbow trout (*Oncorhynchus mykiss*) in Denmark as well as in other European countries. Both RTFS and CWD are caused by *Flavobacterium psychrophilum.* In Denmark it was estimated that RTFS in 1998 caused the death of 88 million fry yielding a financial loss of 18 million DKK (approximately 2.5 million Euro) [1]. Outbreaks usually occur at water temperatures of 8-14°C with mortalities of 50-60%. Infected fry exhibit lethargy, loss of appetite, ascites and anaemia. In fingerlings and larger fish the disease is referred to as coldwater disease (CWD) and causes mortalities of 2-10%, mostly at water temperatures of 2-10°C. The affected fish show ulcers and may suffer from blindness.

At present, RTFS can be controlled by the antimicrobial agent florfenicol. Varying resistance patterns in *F. psychrophilum* to the licensed drugs oxolinic acid and trimethoprim/sulfadiazine are seen and the use of these drugs cannot be recommended [2]. A total of 387 strains isolated in the period 1994-1998 were tested with the antimicrobial agents used in Denmark using an agar dilution method [3]. Sixty strains from the last 4 years (2006 -2009) have been tested in an ongoing project by the same methods to evaluate if the resistance pattern had changed during the last decade. The preliminary results showed that the mean MIC values concerning the tested antibiotics had changed. A higher level of MIC values of both oxolinic acid and trimethoprim/sulfadiazine were found, and a possible explanation might be that the drugs are used for treatment of other bacterial diseases. In contrast a lower level of MIC values of oxytetracycline and amoxicillin were seen, both drugs have not been used for treatment since the mid 1990s. Since 1996 florfenicol has been the drug of choice and a higher mean MIC level was found compared to the earlier study, which indicate that resistance problems might occur in the future.

No registered, commercial vaccines against infections caused by *F. psychrophilum* are available. There is, therefore, a need for alternative treatments of *F. psychrophilum* infections in aquaculture, especially in infected fry. Bacteriophage control of this bacterium may constitute a realistic approach in the treatment of RTFS and CWD. However, a detailed understanding of phage properties and phage-host interactions is needed to evaluate the potential of *F. psychrophilum* bacteriophages. For that purpose 22 *F. psychrophilum* phages from Danish rainbow trout farms were isolated and characterized [4]. The phage genome sizes differed considerably and fell into three major size classes (8.5 to 12 kb, 48 kb and 90 kb). Phage host ranges comprised from 5 to 23 of the 28 tested *F. psychrophilum* strains, and 18 of the phage isolates showed unique host ranges. Each bacterial strain had a unique susceptibility pattern to the 22 phages. In general, the characterization documented the presence of diverse *F. psychrophilum* phage communities in trout farms with highly variable patterns of infectivity. The characterization of broad host range phages with strong lytic potential against numerous pathogenic *F. psychrophilum* host strains thus provides the foundation for future exploration of their

potential in the treatment of RTFS and CWD.

An experiment was set up to investigate the occurrence of bacteriophages and bacteria in rainbow trout during the initial stages of an infection [5]. The fish were either injected intraperitoneally with phages, bacteria or both. Samples were taken from each tank immediately after the fish had been injected, and thereafter with regular intervals. From each fish, samples from spleen, kidney, brain and peritoneal cavity were taken and plated onto TYES (tryptone yeast extract salts) agar for the isolation of the bacterium, whereas samples from spleen, kidney and brain were preserved in SM-buffer with chloroform for the studies on the presence of the phage by using plaque-assays. The results of this experiment showed that the phage and the bacterium were still found in some of the fish 10 days after infection, both in the group only injected with phages as well as in the group injected with phages and bacteria. The results indicate the potential for phage-therapy of *F. psychrophilum* but further studies are needed to clarify the right conditions for a successful treatment.

Prevention of the disease by proper stock management and husbandry is important. Hatcheries based entirely on ground water recirculation systems and good management procedures have been shown to be a possible method for hatcheries to avoid disease outbreaks with *F. psychrophilum* [6]. A similar trend was expected from a project combining increased production on rainbow trout farms and reduced environmental impact. Eight traditional flow-through farms have been redesigned to “model farms” based on recirculation technology. *F. psychrophilum* was isolated from fish on all eight farms mainly from gills and skin mucus, but also from ulcers and internal organs [7]. The significance of the occurrence of *F. psychrophilum* not in combination with disease outbreak is unclear. There were, however, indications that outbreaks of CWD had been a problem during winter time. The results obtained in the monitoring period yielded an important basis for studies to further improve our knowledge of infections caused by *F. psychrophilum*.

Methods used for characterization of *F. psychrophilum* have been biotyping, serotyping, plasmid profiling and ribotyping [2; 8]. Previous investigations of Danish isolates showed a high degree of similarity, which might indicate the use of other genotypic typing methods with a higher level of discrimination power. Results obtained by pulsed field gel electrophoresis (PFGE) with other bacterial species have proved excellent for typing purposes. Recent published papers describe the use of PFGE techniques to examine *F. psychrophilum* diversity [9;10]. Our preliminary results suggest a clonal genetic structure with slight differences among the types also seen by serotyping and ribotyping. However, this study also showed that when comparing isolates from the gills, skin and internal organs, respectively, two different clonal types were seen using an 80% similarity cut off value. It might be suggested that one of the clones contain the virulent isolates whereas the other the non-virulent isolates, but additional studies as well as further interpretation of the PFGE data are necessary for confirming this hypothesis.

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